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Can Use of Viral Load Improve Norovirus Clinical Diagnosis and Disease Attribution?

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Background. Real-time reverse-transcriptase polymerase chain reaction (RT-PCR) is the state-of-the-art diagnostic for norovirus. Cycle threshold (Ct), an indicator of viral load, may be associated with symptomatic disease as well as demographic and outbreak characteristics.

Methods. Data on (1) outbreak and sporadic cases and (2) asymptomatic controls in the United States and Latin America were analyzed. With multivariate regression models, we assessed relationships between various factors and Ct values, and we calculated odds ratios (ORs) for the presence of symptoms and attributable fractions of norovirus. Receiver-operating characteristic analysis was performed to define an optimal Ct cutoff to identify disease-causing infections.

Results. Cycle threshold values were lower (ie, higher viral loads) among symptomatic cases (model-adjusted mean \pm standard error: 25.3 ± 1.2) compared with asymptomatic controls (28.5 ± 1.4). Cycle threshold values were significantly different across age groups, norovirus genogroups, timing of specimen collection, outbreak settings, and transmission modes. Genogroup II (GII) Ct values were associated with presence of symptoms (OR = 1.1), allowing us to estimate that 16% of diarrheal disease was attributable to norovirus. The optimized Ct cutoff led to poor sensitivity and specificity for genogroup I and GII.

Conclusions. Cycle threshold values were associated with host, pathogen, and outbreak factors. Cycle threshold values may not effectively distinguish disease-causing infection for individual patients, but they are useful for epidemiological studies aiming to attribute disease.

Keywords. attributable fractions; cycle threshold; norovirus; real-time PCR; receiver-operating characteristic analysis.

Norovirus affects people from all age groups and is associated with approximately one fifth of acute gastroenteritis (AGE) cases worldwide [1]. The World Health Organization estimated that norovirus caused 684 million diarrheal illnesses and 212 000 deaths in 2010 [2]. Reverse-transcriptase polymerase chain reaction (RT-PCR) assays are the most commonly used diagnostic methods for surveillance, outbreak investigations, and research, and this has allowed for more robust estimates of the norovirus disease burden [3]. RT-PCR assays can detect norovirus at low levels in stool specimens (ie, high “analytical” sensitivity and specificity), and, because the virus can be shed for a prolonged period of time, the virus can be detected in

samples from asymptomatic individuals. Using RT-PCR, norovirus can be detected in 7% of individuals without AGE symptoms compared with 18% with symptoms [1]. Hence, detection of virus does not necessarily indicate that norovirus was a cause of disease, and the “clinical” sensitivity and specificity of RT-PCR assays (ie, identification of disease-causing etiology) are debatable.

The cycle threshold (Ct) value of real-time RT-PCR assays, which is the number of PCR cycles required for the fluorescent signal to cross the threshold, has been used to determine disease etiology for some pathogens [4–6]. The Ct value is inversely proportional to the amount of viral nucleic acid in specimens (ie, lower Ct values indicate higher amount of virus), so it can be used as a proxy of viral load. For rotavirus A, symptomatic cases have significantly higher viral loads than asymptomatic controls, and the optimal Ct value cutoff can be selected to distinguish disease-causing infection [5, 6]. For norovirus, in contrast, studies showed the same/similar concentrations or detection rates between symptomatic cases and asymptomatic controls [7–9]. Even if there was a significant difference in norovirus viral load between cases and controls, the distribution of viral load substantially overlapped [10–12]. Because of these issues, it is not clear whether higher concentrations of norovirus

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represent symptomatic infection nor whether the norovirus Ct value should be considered for clinical diagnostic purposes.

In addition to individual clinical diagnoses, some studies have attempted to use Ct values to estimate attributable fraction (AF) of norovirus-related illness [13, 14]. However, it has been poorly understood how Ct values vary across host and pathogen characteristics and outbreak and logistical factors, which could affect these attribution analyses.

To fill these gaps, we assessed how norovirus Ct values of human clinical specimens are associated with the presence of symptoms as well as demographic and outbreak characteristics. We also attempted to quantify an optimal cutoff for the Ct value to differentiate symptomatic from asymptomatic norovirus infections.

METHODS

Study Sources

We extracted laboratory and epidemiological data from 3 study sources (Supplementary Table 1). The first study source included data from norovirus outbreaks collected through CaliciNet, a national norovirus outbreak surveillance network of public health laboratories in the United States coordinated by the Centers for Disease Control and Prevention [15]. Outbreak specimens are tested by real-time RT-PCR for norovirus by CaliciNet certified laboratories. Positive specimens are genotyped by sequencing [16]. CaliciNet laboratories do not test specimens from asymptomatic controls.

The second study source constitutes data from 2 population-based studies conducted in Latin America. Becker-Dreps et al [17] tested stool specimens from sporadic diarrhea patients <5 years of age in randomly selected households in Nicaragua. They also collected stool specimens from healthy controls who were matched by sex, age group, and neighborhood with the diarrhea cases. In Ecuador, Lopman et al [18] conducted a birth cohort study to estimate the incidence of norovirus gastroenteritis. They enrolled healthy children <2 weeks of age, and they routinely collected stool specimens from all children from birth to 3 years.

The third study source includes data from routine surveillance of AGE and healthy controls enrolled through the New Vaccine Surveillance Network (NVSN) from 2008 to 2012. Active, prospective surveillance methods have been previously published [19] and include enrollment of children <11 years of age having diarrhea and/or vomiting and seen in a hospital, emergency department, or outpatient clinic in 1 of 7 large medical institutions throughout the United States. Healthy controls had no diarrhea or vomiting in the 2 weeks preceding their enrollment at well child visits at these medical institutions.

Norovirus Real-Time Reverse-Transcriptase Polymerase Chain Reaction and Genotyping

All studies used the same norovirus real-time RT-PCR protocol as described previously [15, 17–19]. Positive samples were then genotyped by Sanger sequencing using conventional RT-PCR

products, and sequences were genotyped by comparing them with norovirus reference sequences [15].

Rotavirus Testing Methods

Stool samples were tested for rotavirus by RT-PCR or by a commercial enzyme immunoassay (Rotaclone; Meridian Bioscience or Oxoid, Cambridge, United Kingdom) in the Latin America studies and NVSN [17, 19].

Assessing Associations With Cycle Threshold Values

We built Poisson regression models to assess relationships between norovirus Ct values and demographic, clinical, and outbreak characteristics. We fit multivariate models to control for potential confounders, because it has been known that demographic, clinical, and outbreak characteristics are closely related to each other. For example, many of the elderly, especially women, live in long-term-care facilities where norovirus outbreaks frequently occur [20], and specimens may be collected earlier in these settings.

A total of 4 models were built for different objectives as described in Table 1. In the first model (Model 1), we included sex, age, presence of AGE symptoms, month of symptom onset, and norovirus genotype as independent variables. Because the date of symptom onset was unavailable for the symptomatic cases in the Ecuador dataset, month of specimen collection was used as a proxy of month of illness onset. For asymptomatic controls, month of specimen collection was used to indicate timing. Model 1 was fit with each of the 3 studies separately. In addition, it was fit with a combined dataset of these 3 studies including a “study” as a random effect and other factors as fixed effects. The second model (Model 2) was created by adding the timing of specimen collection (ie, days between symptom onset and specimen collection) to Model 1 to evaluate its relationship with norovirus Ct values. Because asymptomatic controls did not have symptom onset, Model 2 was fit only with symptomatic cases in each study source as well as in the combined dataset, and the presence of AGE symptoms was removed from the model. The third model (Model 3) was created by adding outbreak characteristics (ie, transmission mode and outbreak setting) to Model 2 to assess how they are associated with norovirus Ct values. We fit Model 3 with outbreak data collected through CaliciNet. Lastly, the fourth model (Model 4) was built by adding rotavirus testing results to Model 1 to evaluate whether coinfection with rotavirus was associated with norovirus Ct values. Model 4 was fit with the Latin America studies and NVSN as well as a combined dataset of these 2. CaliciNet was not included in Model 4 because rotavirus testing results were not available. With these 4 models, we calculated adjusted means of norovirus Ct values in each subgroup using the pseudo-likelihood technique. We also performed all-possible pairwise comparisons of adjusted means using Tukey’s method, which correct for multiple testing [21].

When fitting these models, we could not include some records because of their missing information on variables. Most importantly,

Table 1. Objectives and Variables, Study Sources, and Subjects Included in Four Multivariate Poisson Regression Models

Model Characteristics	Model 1	Model 2	Model 3	Model 4
Objectives	To assess how host and virus factors may affect norovirus Ct values	To assess how the timing of specimen collection may affect norovirus Ct values among symptomatic cases	To assess how outbreak characteristics may affect norovirus Ct values	To assess how rotavirus coinfection may affect norovirus Ct values
Variables				
Sex	✓	✓	✓	✓
Age	✓	✓	✓	✓
Presence of AGE symptoms	✓			✓
Month of symptom onset	✓	✓	✓	✓
Norovirus genotype (or genogroup)	✓	✓	✓	✓
Days between symptom onset and specimen collection		✓	✓	
Transmission modes			✓	
Outbreak setting			✓	
Rotavirus testing result				✓
Study sources				
CaliciNet	✓	✓	✓	
Latin America studies	✓	✓		✓
NVSN	✓	✓		✓
Subjects	Both symptomatic cases and asymptomatic controls	Symptomatic cases only	Symptomatic cases only	Both symptomatic cases and asymptomatic controls

Abbreviations: AGE, acute gastroenteritis; Ct, cycle threshold; NVSN, New Vaccine Surveillance Network.

50% of the records in the CaliciNet data were not used in the models, mainly because of missing date of symptom onset (see Results). To see whether the missing data had any significant effects, we ran Model 1 with datasets created by multiple imputation and found that none of the estimates appreciably changed after imputation. Therefore, we used the models with only complete data. All analyses were performed with SAS 9.3 (SAS Institute, Cary, NC).

Association With Norovirus Disease and Attributable Fraction

We estimated odds ratios (ORs) from multivariate logistic regression models, where the outcome was case-control status and the predictor was an inverse measure of norovirus Ct value (Cq), which was calculated as follows: $Cq = 41 - Ct \text{ value}$. For this analysis, Ct values for negative specimens (ie, specimens whose fluorescent signals did not cross the threshold by the last PCR cycle) were set to be 41 and their Cq values were set to be 0. To control for potential confounders, we included the detection of norovirus (positive vs negative), patients' age, sex, and month of symptom onset in the model. Due to the sparse data, we removed variables by backward elimination ($\alpha = 0.05$) and fit the model separately with each study. To attribute the proportion of AGE cases to norovirus in each study, we also calculated population AFs by summing attributions of norovirus-related illness across each of the Cq values.

$$\sum AF_i, \text{ where } AF_i = \Pr(Cq_i | \text{cases}) * (1 - \frac{1}{OR_i})$$

where $\Pr(Cq_i | \text{cases})$ is the proportion of i^{th} Cq value among symptomatic cases. Confidence intervals (CIs) were estimated

by bootstrap with 1000 iterations. The models were run separately for genogroup I (GI) and genogroup II (GII). The CaliciNet data were not included in these analyses, because all its data are from symptomatic subjects.

Receiver-Operating Characteristic Analysis

We performed receiver-operating characteristic (ROC) analysis with the aim of defining an optimal Ct cutoff to distinguish symptomatic norovirus infections and asymptomatic infections. We defined a reference positive group and reference negative group based on the presence of AGE symptoms and timing of specimen collection. Reference positive included specimens collected from symptomatic cases within 3 days of symptom onset. Reference negative included specimens collected from asymptomatic controls regardless of the timing of specimen collection. All specimens, including reference negatives, were positive for norovirus by real-time RT-PCR and confirmed by sequencing. We conducted ROC analyses separately for norovirus GI and GII. The Youden index (sensitivity+specificity-1) was used to identify an optimal cutoff for the Ct value [22–24].

RESULTS

Our dataset included 12 875 clinical specimens from CaliciNet, 659 from the Latin America studies, and 3997 from NVSN. Of these, norovirus Ct values were reported for 12 039 (94%) in CaliciNet, 227 (34%) in the Latin America studies, and 644 (16%) in NVSN. The remaining was norovirus negative by real-time RT-PCR. Median age was 77 years in CaliciNet, 2 years in

the Latin America studies, and 1 year in NVSN. Norovirus GI Ct values were lowest (median, 24; range, 6–43) in CaliciNet, followed by NVSN (median, 27; range, 16–40) and the Latin America studies (median, 29; range, 19–38) (Figure 1). For GII, CaliciNet (median, 22; range, 6–43) and NVSN (median, 22; range, 10–39) had lower Ct values compared with the Latin America studies (median, 28; range, 16–38). Lower Ct values were observed among symptomatic cases (GI: median 24, range 6–43; GII: median 22, range 6–43) compared with asymptomatic controls (GI: median 28, range 19–40; GII: median 28, range 15–38), but the distributions of Ct values substantially overlapped between the 2 groups (Figure 1).

Of 12910 norovirus-positive specimens, Model 1 included 5989 (50%) from CaliciNet, 194 (85%) from the Latin America studies, and 585 (91%) from NVSN. The remaining records were not used in Model 1, because both norovirus GI and GII were detected by RT-PCR from the same specimens ($n = 40$), because of the missing information on the factors included in Model 1 ($n = 6086$), or both ($n = 16$).

After controlling for other covariates, a number of factors were found to have significant associations with norovirus Ct values. In Model 1 (Table 2), norovirus Ct values were significantly lower among symptomatic cases (model-adjusted mean \pm standard error: 25.3 ± 1.2) than asymptomatic controls (28.5 ± 1.4 , $P < .0001$) (Figure 2A). Lower Ct values were found in specimens from young children and the elderly compared with the middle age group ($P < .0001$) (Figure 2C). We also found a significant

association between the month of illness onset and Ct value in the combined dataset ($P = .03$), but the difference was relatively small and not significant in the individual study sources (Supplementary Figure 1C and Supplementary Table 2). Variation in Ct values was observed across norovirus genotypes ($P < .0001$) (Supplementary Figure 1D); overall, specimens positive for norovirus GII had lower Ct values (26.2 ± 1.1) than specimens positive for GI (27.4 ± 1.1 , $P < .0001$) (Figure 2B). We did not find any evidence of an association of patients' sex with Ct values (Supplementary Figure 1A).

In Model 2 with symptomatic cases, the Ct values were significantly higher when specimens were collected >7 days after illness onset (range, 26.4–27.5), compared with those collected within 7 days (range, 24.5–25.0; $P < .0001$) (Table 3 and Figure 2D). In Model 3, specimens from outbreaks involving transmission by food or unknown/other modes had significantly lower Ct values (22.6 ± 0.7 and 22.8 ± 0.7 , respectively) than those having person-to-person transmission (23.3 ± 0.6 , Tukey-adjusted $P < .05$). Among all the different outbreak settings, specimens from cruise ship outbreaks had the lowest Ct values (20.8 ± 0.9). Healthcare facilities had lower Ct values (23.0 ± 0.9) than child care facilities (25.0 ± 1.0) and schools (24.5 ± 0.9 , Tukey-adjusted $P < .05$). According to Model 4, coinfection with rotavirus was not significantly associated with the norovirus Ct value (Supplementary Figure 1B).

Norovirus GII Cq value was associated with presence of disease symptoms (OR = 1.13 (95% CI, 1.05–1.21) in Latin America and OR = 1.13 (95% CI, 1.08–1.18) in NVSN, Table 4

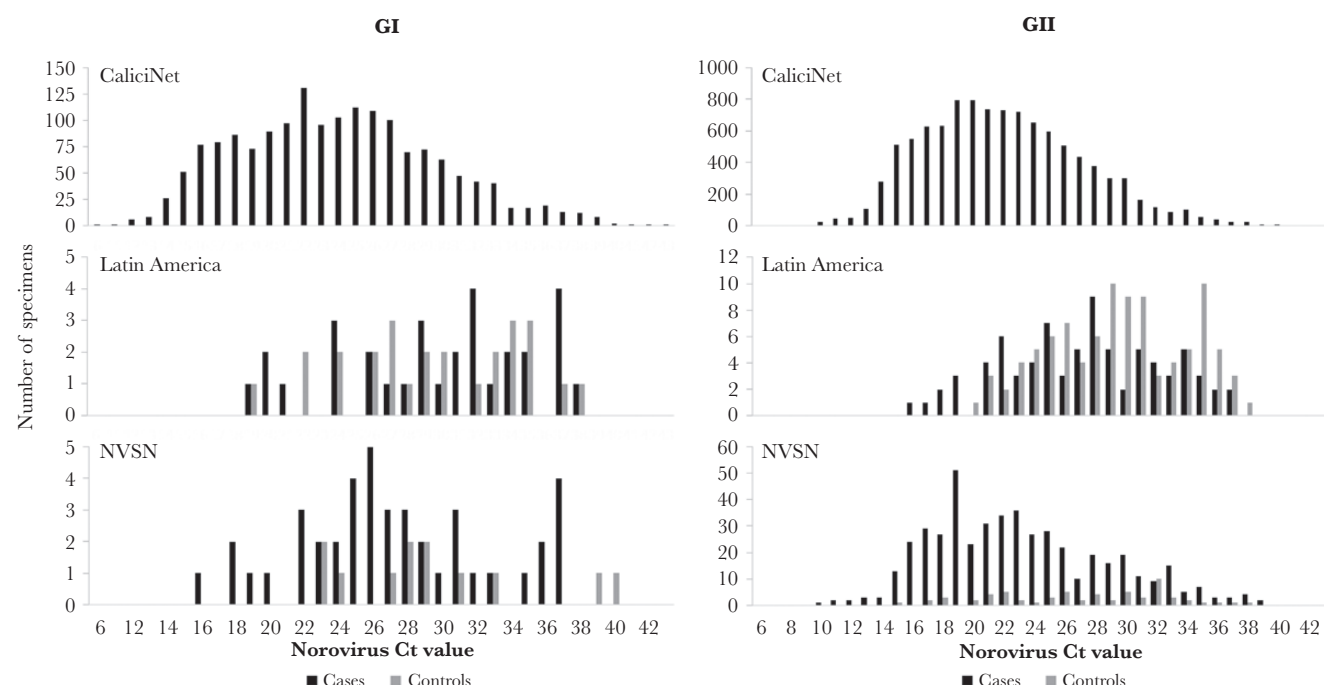


Figure 1. Distribution of norovirus Ct value by Study and by the presence of AGE symptoms. *Specimens infected with both GI and GII and specimens in which norovirus infection was not confirmed by sequencing are not included in these graphs. Abbreviations: AGE, acute gastroenteritis; NVSN, New Vaccine Surveillance Network; Ct value, cycle threshold value.

Table 2. Mean Ct Values Estimated by the Multivariate Mixed-Effect Poisson Regression Model (Model 1)

Characteristics	Number of Specimens	Mean Estimate	(95% CI)	P Value
Sex				.06
Male	2548	26.7	(24.4–29.3)	
Female	4220	27.0	(24.6–29.6)	
Age group				<.0001
<1 yr	260	25.8	(23.5–28.4)	
1 to <2 yr	340	26.2	(23.8–28.7)	
2 to <3 yr	174	26.7	(24.3–29.3)	
3 to <4 yr	89	26.6	(24.1–29.4)	
4 to <5 yr	36	28.7	(25.7–32.1)	
5 to <10 yr	148	27.9	(25.3–30.8)	
10 to <18 yr	149	28.1	(25.5–31.1)	
18 to <45 yr	837	28.1	(25.5–30.9)	
45 to <65 yr	907	27.0	(24.5–29.7)	
65 to <75 yr	573	26.5	(24.1–29.2)	
75 to <85 yr	1114	25.9	(23.5–28.5)	
85 to <95 yr	1814	26.0	(23.6–28.5)	
≥95 yr	327	26.0	(23.6–28.7)	
Cases vs controls				<.0001
Controls	173	28.5	(25.9–31.4)	
Cases	6595	25.3	(23.1–27.8)	
Month of onset				.03
January	1274	26.7	(24.3–29.3)	
February	1192	26.6	(24.2–29.1)	
March	1076	26.9	(24.5–29.5)	
April	597	26.6	(24.2–29.2)	
May	414	27.5	(25.0–30.2)	
June	235	26.6	(24.2–29.3)	
July	196	27.3	(24.8–30.1)	
August	120	26.6	(24.2–29.4)	
September	159	26.7	(24.3–29.5)	
October	218	27.8	(25.3–30.7)	
November	457	26.3	(24.0–28.9)	
December	830	26.7	(24.3–29.3)	
Norovirus genotype				<.0001
GI.1	11	30.9	(26.8–35.6)	
GI.2	81	26.3	(23.9–29.0)	
GI.3	371	25.6	(23.4–28.0)	
GI.4	67	28.8	(26.1–31.9)	
GI.5	45	30.5	(27.4–33.8)	
GI.6	247	30.0	(27.4–32.8)	
GI.7	39	23.3	(20.9–26.1)	
GI.8	8	28.9	(24.6–33.9)	
GI.9	4	33.6	(27.4–41.3)	
GI untypeable	1	24.1	(15.4–37.8)	
GII.1	331	28.4	(26.0–31.1)	
GII.2	133	26.1	(23.7–28.6)	
GII.3	84	26.2	(23.7–28.9)	
GII.4_DenHaag	352	26.3	(24.0–28.8)	
GII.4_NewOrleans	1825	26.6	(24.3–29.0)	
GII.4_Osaka	14	27.6	(24.0–31.7)	
GII.4_Sydney	2256	25.8	(23.6–28.2)	
GII.4_Yerseke	2	23.0	(16.9–31.4)	
GII.4_Untypeable	10	27.9	(23.9–32.6)	
GII.5	27	28.6	(25.5–32.2)	
GII.6	382	24.9	(22.8–27.3)	
GII.7	167	24.3	(22.1–26.6)	
GII.8	3	24.6	(18.9–32.1)	
GII.12	108	28.7	(26.1–31.6)	
GII.13	75	24.0	(21.8–26.6)	
GII.14	34	23.9	(21.4–26.7)	
GII.15	1	18.3	(11.1–30.2)	
GII.16	2	25.5	(18.6–34.8)	
GII.17	26	24.9	(22.1–28.1)	
GII.21	2	35.4	(27.1–46.2)	
GII.23	6	30.0	(25.4–35.5)	
Unable to confirm	54	33.4	(30.3–36.9)	

Abbreviations: CI, confidence interval; Ct, cycle threshold; GI, genogroup I; GII, genogroup II.

and Supplementary Figure 2), but GI Cq was not (OR = 1.01 (95% CI, 0.91–1.13) in Latin America and OR = 1.07 (95% CI, 0.95–1.21) in NVSN). Based on this relationship, 16.0% and 16.2% of diarrheal disease in the Latin America studies and NVSN, respectively, could be attributed norovirus GII.

According to the ROC analysis, the optimal Ct value cutoff with the highest Youden index was 26 for GI and 25 for GII (Figure 3 and Supplementary Table 3), but both sensitivity (GI, 73%; GII, 76%) and specificity (GI, 65%; GII, 68%) were poor.

DISCUSSION

We found that host (AGE symptoms and age), pathogen (norovirus genotype), outbreak (transmission mode and setting), and logistical (timing of specimen collection) factors were significantly associated with norovirus real-time RT-PCR Ct values. The estimated mean Ct value was lower among symptomatic cases than controls, after controlling for other factors in the Poisson regression models. However, the distributions overlapped between the symptomatic and asymptomatic individuals, and, therefore, there was no clear Ct value cutoff that differentiates symptomatic and asymptomatic infections with high clinical sensitivity and specificity.

We found higher Cq to be associated with disease symptoms. Using the model coefficients, we were able to estimate the fraction of diarrhea that can be attributed to norovirus. As expected, these fractions (16.0% in the Latin America studies and 16.2% in NVSN) were lower than the prevalence of norovirus (20.8% in the Latin America studies and 19.2% in NVS). Studies without healthy controls may be able to apply our estimated ORs to their Ct value data and calculate AFs of norovirus for their study.

Not only the presence of symptoms but also other factors were found to have significant associations with norovirus Ct values. Young children and the elderly had higher viral load than those from the middle age group potentially as a result of lack of prior exposures to norovirus among young children or immune senescence of the elderly. Most of the people in the middle age group, in contrast, might have some degree of acquired immunity against norovirus from previous exposure. The relationship between the timing of specimen collection and Ct value highlights the importance of early specimen collection for diagnostic purposes. The Ct values were significantly lower in specimens that were collected within 7 days after the symptoms onset. This confirms the recommendations to collect stool samples as early as possible after onset of symptoms preferably within 1 week [25]. We found that GII-positive specimens had slightly lower Ct values compared with GI-positive samples, but overall Ct values varied across different genotypes even among viruses belonging to the same genogroups. This may be due to real differences between genotypes but more likely is caused by the difference in sensitivity of the PCR assays for the different genotypes, suggesting that the Ct values need to be interpreted

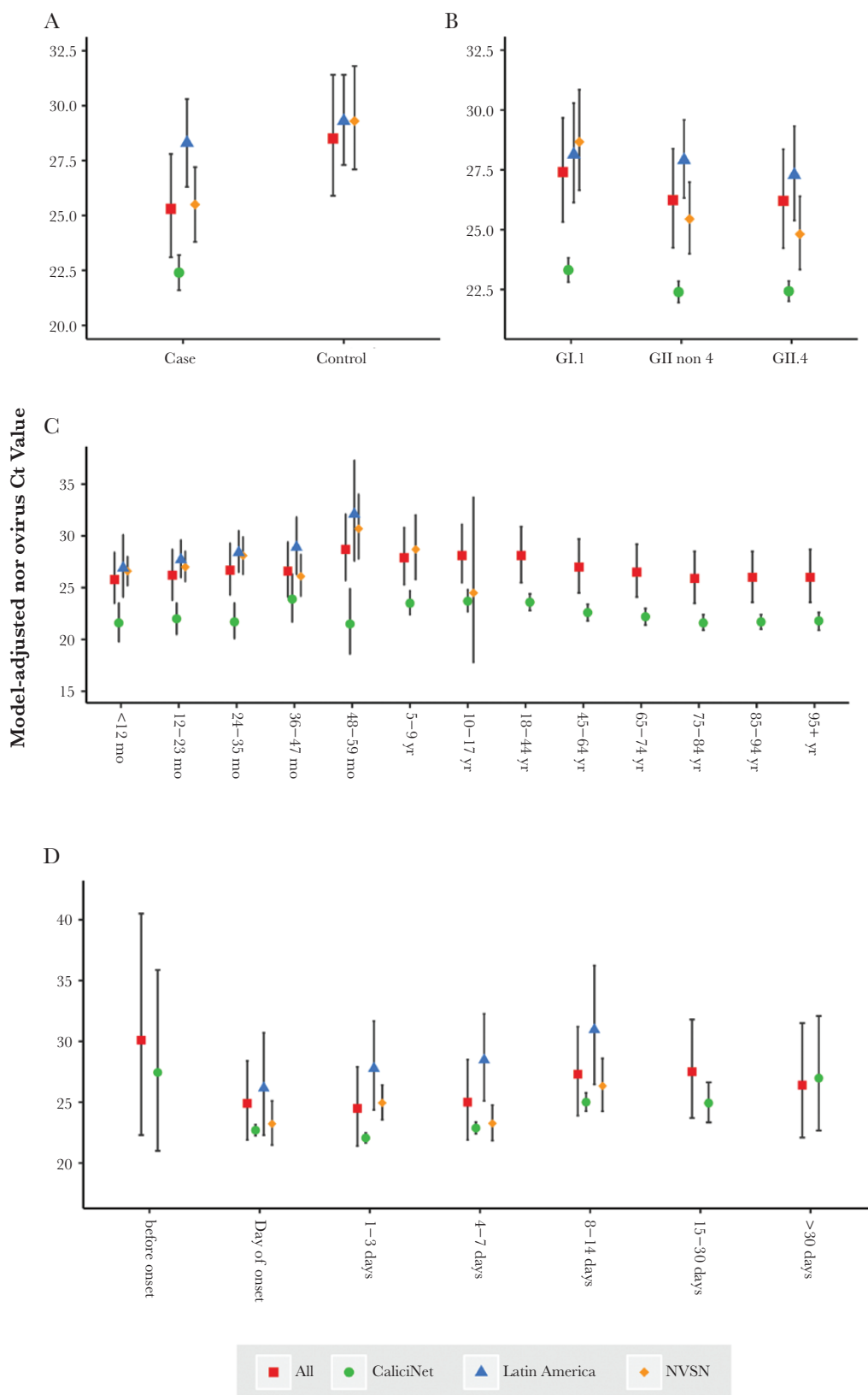


Figure 2. Distribution of norovirus Ct values estimated by the multivariate Poisson regression models. Dots represent point estimates of the model-adjusted norovirus Ct values, and bars represent their 95% CIs. A: Presence of AGE symptoms (Model 1); B: Norovirus genogroups (Model 1); C: Patient age (Model 1); D: Timing of specimen collection (Model 2). Abbreviations: Ct value, cycle threshold value; CI, confidence interval; NVSN, New Vaccine Surveillance Network; GI, genogroup I; GII, genogroup II; mo, months; yr, year.

Table 3. Mean Ct Values Estimated by the Multivariate Mixed-Effect Poisson Regression Model by Timing of Specimen Collection, Outbreak Characteristics, and Rotavirus Coinfection (Model 2–4)

Characteristics	Number of Specimens	Mean Estimate	(95% CI)	PValue
Specimen collection (Model 2)				<.0001
Before symptom onset	2	30.1	(22.3–40.5)	
Date of symptom onset	2270	24.9	(21.9–28.4)	
1–3 days after symptom onset	2738	24.5	(21.4–27.9)	
4–7 days after symptom onset	1184	25.0	(21.9–28.5)	
8–14 days after symptom onset	283	27.3	(23.9–31.2)	
15–30 days after symptom onset	38	27.5	(23.7–31.8)	
>30 days after symptom onset	12	26.4	(22.1–31.5)	
Transmission mode (Model 3)				.002
Environmental	8	26.3	(22.6–30.5)	
Foodborne	852	22.6	(21.3–24.0)	
Person-to-person	3966	23.3	(22.0–24.7)	
Waterborne	13	23.0	(20.3–26.1)	
Unknown/other modes	1150	22.8	(21.5–24.1)	
Outbreak setting (Model 3)				<.0001
Child care facility	93	25.0	(23.1–27.0)	
Correctional facility	83	23.1	(21.3–25.1)	
Cruise ships	76	20.8	(19.1–22.7)	
Food facility	607	24.0	(22.4–25.7)	
Healthcare facility	215	23.0	(21.3–24.7)	
Long-term care facility	3862	23.5	(21.9–25.2)	
School	347	24.5	(22.8–26.2)	
Other setting	670	23.7	(22.1–25.3)	
Unknown setting	36	24.8	(22.5–27.3)	
Rotavirus (Model 4)				.3
Positive	54	29.2	(26.4–32.4)	
Negative	719	28.3	(25.9–30.9)	

Abbreviations: CI, confidence interval; Ct, cycle threshold.

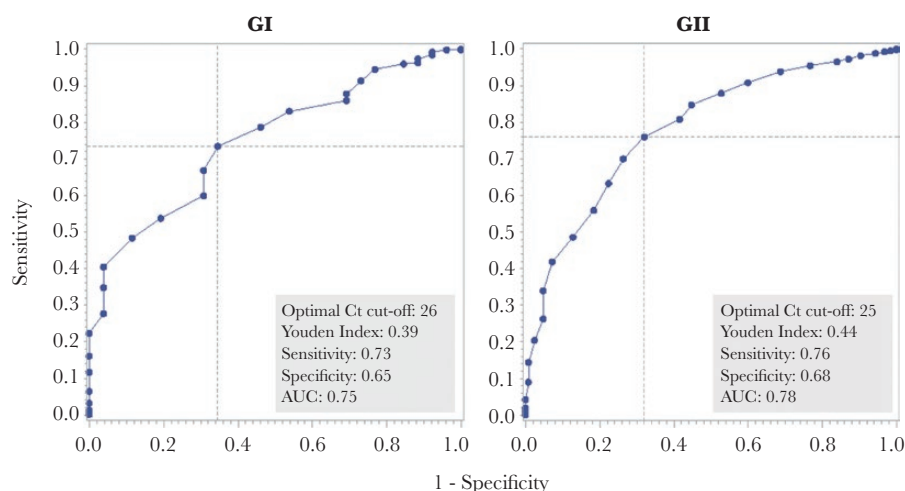
Table 4. Association Between the Pathogen Quantity and Presence of Symptoms and Attributable Fractions^a

Norovirus Genogroup and Study	Odds Ratios per 1-Unit Increase in Cq Value (95% CI; P Value)	Attributable Fractions
Norovirus GI		
Latin America	1.01 (0.91–1.13; <i>P</i> = .8)	1.1%
NVSN	1.07 (0.95–1.21; <i>P</i> = .3)	0.9%
Norovirus GII		
Latin America	1.13 (1.05–1.21; <i>P</i> = .001)	16.0%
NVSN	1.13 (1.08–1.18; <i>P</i> < .0001)	16.2%

Abbreviations: CI, confidence interval; Cq, inverse measure of norovirus Ct value which was calculated as $Cq = 41 - Ct \text{ value}$; Ct, cycle threshold; GI, genogroup I; GII, genogroup II; NVSN, New Vaccine Surveillance Network.^aAll models except GI in the Latin America studies controlled for the detection of norovirus (positive vs negative), patients' age, sex, and month of symptom onset. The model for GI in the Latin America studies included the detection of norovirus and month of symptom onset after backward elimination.

carefully. Another explanation could be that the Ct differences can be attributed to differences in genetic susceptibility against the different genotypes [26, 27].

Significant relationships were also found between the outbreak characteristics and Ct values. Samples from cruise ship norovirus outbreaks had the lowest Ct value among all outbreak settings even after controlling for age and the timing of specimen collection. We cannot see a biological basis for lower Ct values in this setting, so we suspect that other unmeasured issues with specimen collection and storage quality may be residually confounding this association. In addition, foodborne outbreaks were found to have lower Ct values compared with person-to-person outbreaks. A potential hypothesis of this finding is that individuals are exposed to higher dose of viruses in foodborne outbreaks compared with person-to-person outbreaks. However, even if exposure doses are higher, a human

**Figure 3.** Receiver-operating characteristic plots for norovirus genogroup I and genogroup II. Abbreviations: GI, genogroup I; GII, genogroup II.

challenge study is not supportive of an association between inoculum dose and viral shedding [28]. Therefore, these findings might be attributable to other unadjusted factors, such as investigation methods (eg, more intense investigations might be implemented for foodborne outbreaks). Further studies are needed to confirm these hypotheses.

An important limitation of our study is that comparing data generated in different laboratories (eg, 32 different CaliciNet laboratories are currently certified) may have affected the Ct data. To address this issue, all staff completed standardized training to become certified to perform the norovirus assays, and laboratories are biennially validated by having them complete a proficiency panel. In addition, mixed-effect models have accounted for correlation among specimens tested in each study. However, these approaches do not perfectly solve the issue. Another limitation is that we could not assess a relationship between Ct values and clinical severity due to the lack of detailed clinical data. A strength of our study is the large sample size, which allowed us to include a number of variables into the models in an attempt to identify the independent effects of host, pathogen, outbreak, and logistical factors. As a result, we found that each of these factors was independently associated with norovirus Ct values after adjustment. The large sample size also helped us identify subtle differences in the Ct value across subgroups, which could have been missed if we had smaller populations.

CONCLUSIONS

In conclusion, we found that the Ct values were associated with presence of symptoms as well as various host, pathogen, outbreak, and logistical factors. The difference in Ct values was subtle and did not lend itself to discriminatory cutoff values for clinical diagnosis of individual specimens; however, those characteristics associated with Ct values could be taken into account for evaluating disease burden or incidence of norovirus.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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